IJFSNPHT

ISSN: 0975 – 8712
IJFSNPHT (2009), 1(1):6-10

The presence of *Salmonella* in food: a challenge to detect it and to improve food safety continously in a global scale

Ignatius Srianta[1], Netty Kusumawati[2], Ira Nugerahani[3], Aysan Husein Juniawati[4] and Elisa Rinihapsari[5]

1 Head of Center for Food and Nutrition Research,
2 Head of Microbiology Laboratory, Food Technology Department,
3 Senior Lecturer of Food Microbiology, Food Technology Department,
4 Research Assistant, Food Technology Department,
Widya Mandala Surabaya Catholic University, Surabaya, INDONESIA
5 Lecturer of Microbiology, School of Pharmacy STIFAR "YAYASAN PHARMASI" Semarang, INDONESIA
srianta_wm@yahoo.com

ABSTRACT

One of major food-borne illness from microorganisms is salmonellosis, which is caused by consumption of food containing *Salmonella*. *Salmonella* responsibles for thousands of cases of food-borne illness yearly. It is considered as one of the most serious threat to human life. The higher incidence of *Salmonella* infections cause higher medical costs and loss of productivity. Cattle, poultry and seafood are food of animal origin that can cause *Salmonella* infection in human. But, some food of non-animal origin can also carry *Salmonella*. Many reports have shown the presence of *Salmonella* in various type of food. Our researches have also shown the presence of *Salmonella* in some food samples i.e. cockle, tiger prawn, squid and egg-fried rice. Among of 39 *Salmonella*-suspect isolates obtained from those samples, 6 isolates similar to *Salmonella paratyphi* A, 3 isolates were similar to *Salmonella typhi* and 5 isolates were similar to *Salmonella enteridis*. The presence of *Salmonella* in food is mostly caused by contamination of polluted water; the improper handling, processing and storage; poor personal hygiene and sanitation; and cross contamination. The globalization lead to the complexity of the food supply chain, providing greater opportunities for contamination and growth of pathogens. Those facts make the challenge to detect the presence of *Salmonella* in food and to improve food safety continously in a global scale become more difficult.

Key words: *Salmonella*, salmonellosis, food-borne illness, challenge, detect, globalization, food safety

INTRODUCTION

Food safety is an increasingly important public health issue. Every person is at risk of food-borne illness. One of the most incidence of food-borne illness from microorganisms is salmonellosis. The general symptoms of salmonellosis are nausea, abdominal pain, drowsiness, diarrhea and a moderate fever, dehydration may occur resulting in great thirst. Salmonellosis is caused by consumption of food containing *Salmonella*. *Salmonella* are Gram negative bacteria, non-sporing rods, usually motile with peritrichus flagella, facultative anaerobes, biochemically characterized by their ability to ferment glucose with the production of acid and gas, and their inability to attack lactose and sucrose. *Salmonella* induce the illness by their death phase following growth in the host's gut and their subsequent lysis with the release of potent endotoxin. This endotoxin forms part of the membrane of the cell and is primarily responsible for the clinical symptoms. This organism can invade the blood stream and thus cause a septicemia and in the worst cases the patient may go into a coma [1,2,3].

Salmonella is responsible for more than 40,000 of cases of food borne-illness every year [1,3]. Approximately 21.5 million infections in year 2000 and 200,000 deaths from typhoid fever globally each year were estimated [4]. Non-typhoid infections cases of 13,271 was notified in Europe during 1997-2002 [5]. Outbreaks associated with *Salmonella* have been reported in African countries [4], Asian countries [6], United States of America [7,8,9,10] and European countries [11,12,13,14].

Salmonella are widely distributed in nature, the primary habitat is intestinal track of animals such as farm animals, reptiles, birds and occasionally insects. Both animal and people can be reservoirs of *Salmonella*. The objectives of this paper is to detect the presence of *Salmonella* in foods which is a never ending threat to human life and a challenge in a global scale.

MATERIALS AND METHODS

Materials

Food samples i.e cockle, tiger prawn, and squid were obtained from markets in Surabaya, Indonesia; egg-fried rice samples were purchased from food vendors in the public transport in Surabaya, Indonesia. Buffer Pepton Water (Merck), Selenite Cystine Broth (Merck), Bismuth Sulphite Agar (Merck) and *Salmonella-Shigella* Agar (Merck), Nutrient Agar (Merck), Peptone Broth (Merck), Simon Citrate (Merck), Kliger Iron Agar (Merck), Semi Solid Sucrose (Merck), Lysine Iron Agar (Merck) and Motility Indole Ornithine (Merck) were purchased from local distributor. Pure culture of *Salmonella*

IJFSNPHT

ISSN: 0975 – 8712
IJFSNPHT (2009), 1(1):6-10

typhi, Salmonella typhimurium, Salmonella parathyphi A and *Salmonella enteridis* were obtained from Food and Nutrition Culture Collection (FNCC) Gadjah Mada University, Yogyakarta, Indonesia.

***Salmonella* detection**

Salmonella detection and isolation referred to Wibowo and Ristanto [15]. The samples were aseptically placed in sterile polyethylene plastic bag, then brought (in cool condition) to the microbiology laboratory. At the time, the presence of *Salmonella* were detected from samples. 25 g of sample was weighed into sterile blending container aseptically. 225 ml of sterile Buffer Peptone Water were added to the sample and blended for 5 minutes. Homogenized mixture was aseptically transfered to sterile wide-mouth jar (500 ml) and left at room temperature for 60 ± 5 minutes with jar securely capped, then incubated at 37°C for 24 hours. The incubated sample were gently shaked, 10 mL of the sample was inoculated to 90 mL of Selenite Cystine Broth, then incubated at 37°C for 24 hours. Then, the incubated sample were gently shaked, 0.1 mL of enriched sample were streaked on Bismuth Sulphite Agar (BSA) and *Salmonella-Shigella* Agar (SSA), then incubated at 37°C for 24 hours. The presence of colonies that may be *Salmonella* were examined. Typical *Salmonella* colonies are brown, gray, or black colonies; sometimes they have a metallic sheen. If typical colonies are present on the BSA and SSA after 24 ± 2 hours incubation, then 1 or more colonies were picked for examining microscopically. Morphological of colony and microscopic characteristic were compared to the positive cultures. All cultures that give similar characteristics to positive cultures should be retained as suspect *Salmonella* isolates and submitted to biochemical test. *Salmonella*-suspect typical colony was purified to obtain the pure culture (isolate) prior to biochemical test. A loopful of colony was streaked on Nutrient Agar then incubated plates 24 hours at 37°C. *Salmonella*-suspect isolates were performed the biochemical tests. Biochemical tests consist of IMVIC test (indole, Methyl Red, Voges-Proskauer, Simon Citrate) and media series test (Kliger Iron Agar, Semi Solid Sucrose, Lysine Iron Agar and Motility Indole Ornithine). The biochemical test result was compared to the positive cultures of *Salmonella typhi, Salmonella typhimurium, Salmonella parathyphi A* and *Salmonella enteridis.*

RESULTS AND DISCUSSION

The presence of *Salmonella* in various foods

The presence of *Salmonella* were detected in those food samples. Table 1 showed 39 of *Salmonella*-suspect isolates obtained from cockle, tiger prawn, squid and egg-fried rice.

Table 1 Result of *Salmonella* detection and isolation of tiger prawn, cockle, squid and egg-fried rice

Sample	Code of *Salmonella*-suspect isolate
Cockle	CS1,CS2,CS3,CS4,CS5,CB1,CB2,CB3,CB4
Tiger Prawn	TS1,TS2,TS3,TS4, TS5,TS6,TB1,TB2,TB3,TB4
Squid	SS1,SS2,SS3,SS4,SS5,SS6,SS7,SB1,SB2, SB3,SB4
Egg-Fried Rice	FS1,FS2,FS3,FS4,FS5,FS6,FB1,FB2,FB3

Note: Code T=Tiger Prawn; C=Cockle; S=Squid; F=Fried Rice; S=*Salmonella-Shigella* Agar; B=Bismuth Sulphite Agar; TS1=Isolate of *Salmonella* from Tiger Prawn using *Salmonella-Shigella* Agar

Table 2 Result of biochemical test of *Salmonella*-suspect isolates from cockle

Code of isolate	IMVIC test				Media series test										
	Indole	MR	VP	SC	KIA			SSS		LIA			MIO		
					S	U	H_2S	U	S	S	U	H_2S	U	S	Indole
CS1	-	-	-	+	K	X	+	K	+	K	A	-	K	+	-
CS2	-	+	-	+	K	A	+	A	+	K	K	-	K	+	-
CS3	-	-	-	+	K	X	+	K	+	K	A	-	K	+	-
CS4	-	-	-	+	K	X	+	K	+	K	A	-	K	+	-
CS5	-	+	-	+	K	A	+	A	+	K	K	-	K	+	-
CB1	-	-	-	-	K	X	+	A	+	K	K	-	K	+	-
CB2	-	-	-	+	K	X	+	A	+	K	K	-	K	+	-
CB3	-	-	-	+	K	X	+	A	+	K	K	-	K	-	-
CB4	+	-	-	+	K	X	+	A	+	K	K	+	K	+	+

Note: code MR, Methyl Red; VP, Voges Proskauer; SC, Simon Citrate; KIA, Kliger Iron Agar; SSS, Semi Solid Sucrose; LIA, Lysine Iron Agar; MIO, Motility Indole Ornithine; S, slant; U, upright; -, negative result; +, positive result; K, alkaline reaction; X, neutral reaction; A, acid reaction.

Results of biochemical test of 9 isolates obtained from cockle were presented in Table 2 (result of other samples were not shown). Other reports also showed the presence of *Salmonella* in cockle, clam, tiger prawn, oyster and seawater [16,17,18,19,20,21,22,23]. *Salmonella* was found in 31.6% of 215 seafood samples, including fish, shrimp, crab, clam, mussel, oyster, squid, cuttlefish and octopus [24].

Three of isolates obtained from cockle (CS1, CS3 and CS4) resulted the similar biochemical reaction to *Salmonella paratyphi A*, but 6 isolates were different from the positive cultures. Cockles are filter feeder whereby seawater is pumped in through the gills which entrap larger planktonic food organisms within the cilia and mucus of the respiratory ephitelium [25]. They may accumulate bacteria present in the surrounding environment. Other reports showed that the most frequently *Salmonella* isolated from cockle were classified to *Salmonella seftenberg, Salmonella typhimurium, Salmonella agona* [21], and *Salmonella typhi* [26]. Among of 10 isolates obtained from tiger prawn, 2 isolates (TS2 and TS3) were similar to *Salmonella typhi*, 3 isolates (TB1, TB3 and TB4) were similar to *Salmonella enteridis* and 1 isolate (TS3) was similar to *Salmonella paratyphi A*. Other researcher also found the presence of *Salmonella* in 0.1% of 1264 frozen tiger prawn samples. It was also found that 846 samples of prawn were contaminated by *Salmonella typhimurium* [17]. In this study, 2 isolates from squid were similar to *Salmonella paratyphi A* and 2 isolates were similar to *Salmonella enteridis*. Other researcher found that squid which caused outbreak were contaminated by *Salmonella oranienburg* [18]. The leading sources of *Salmonella* infections are animals harvested through aquaculture [1]. Food-borne illness reported during 2006 in USA, 18% of 1,270. outbreaks caused by *Salmonella*. Among the 11 deaths, 10 were attributed bacterial etiologies including *Salmonella* serotype *enteridis*. Those cases were related to seafoods commodities including fish, crustaceans and mollusks [27].

Nine isolates of *Salmonella* were found in egg-fried rice samples obtained from food vendors in the public transport. One of them (FS4) was similar to *Salmonella typhi*, but 8 isolates were different from positive cultures. The samples of fried rice consisted of rice, oil, egg, chicken meat, slice of cucumber and tomato, and celery. The most probable source of *Salmonella* was egg and chicken meat, which were animal-origin foods. This is in accordance to the previous epidemiology and environment studies that have implicated eggs and poultry products as primary risk factors for infection [5]. Outbreak caused 38 of 52 people suffered gastrointestinal symptoms after consumed egg-fried rice at Chinese restaurant in Suffolk, United Kingdom. Interviews with the chef revealed that eggs used in the preparation of egg-fried rice, were left at room temperature for seven hours and was used in the preparation for other two rice dishes. According to the epidemiology study, it is strongly suggested the eggs used in the preparation of the egg-fried rice as the vehicle for this outbreak [28]. However, it is not impossible that rice, oil, slice of cucumber and tomato, and celery carry *Salmonella*. *Salmonella* were found in non-animal origin food such as sesame seed products [11], vegetable-coated snack foods [10], potato [29]. Between 1st january 1992 and 31st December 2000, 5.6% of outbreaks of food-borne illness in England and Wales were attributed to salad vegetables and fruit, with *Salmonella* being the most frequent implicated bacterial pathogen [30]. Lettuce has been connected to outbreaks of *Salmonella* serotype *typhimurium* [31].

A challenge to detect *Salmonella* in food

One of the difficulties in the detection *Salmonella* in food is that they are generally present in a very low numbers (<100 cfu/g) in the midst of up to million or more other bacteria. These microbes might be lost among a background of indigineous microbes, and substances in the foods themselves may hinder recovery [31]. Consequently, non-selective pre-enrichment and selective enrichment are needed to resolve the problem.

The conventional method usually include multiple subcultures and biotype or serotype-identification step [31]. The conventional method, consists of non-selective pre-enrichment using Buffer Peptone Water and incubated at 37^{o}C for 18-24 hours; selective enrichment using Selenite Cysteine Broth and incubated at 37^{o}C for 18-24 hours; the selective enrichment culture is usually inoculated on to at least two selective agar media and incubated at 37^{o}C for 24 hours. A number of selective chromogenic agar media specifically designed for the differentiation of *Salmonella* colonies are commercially available. Typical *Salmonella* colonies on selective agar are subcultured onto non-selective media prior to confirmatory testing [32]. This method are highly cost, laborious and time-consuming (requiring up to 5 days to obtained confirmed result). Whereas, the demand of food industry and the public is a cost-efficient, labor-efficient and rapid method. To address this challenge, a substantial number of alternative rapid methods have been developed: PCR(polymerase chain reaction)-based [31, 33, 34], lightcycler system [35], ELISA [36], combination of immunomagnetic separation and PCR [37]. Almost all rapid test protocols include a selective enrichment stage, and then apply rapid detection techniques to replace culture on selective agars and further confirmatory tests. Most can claim to produce a result in approximately 48 hours or less, depending on the enrichment protocol. Josefsen et al.

[33] improved a 12 hours *Salmonella* detection method, based on 8 hours of pre-enrichment followed by automated DNA extraction and a sensitive real-time PCR. Many of these methods have been successfully validated by the AOAC.

A challenge to improve food safety continously in a global scale

The presence of *Salmonella* in food mostly caused by contamination of polluted water; the improper handling, processing and storage; poor personal hygiene and sanitation; and cross contamination. It is a challenge for food industries and governments to improve food safety continously. Global trade in food, urbanization, changes in lifestyles, international travel and environmental pollution cause the food production chain has become more complex, providing greater opportunities for contamination and growth of pathogens. Those facts make the challenge for food industries and governments to improve the food safety become more difficult. Implementation of Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP) and Hazard Analysis and Critical Control Point (HACCP) are requirement for food processing industries to produce safe food products and also for food service industries to serve safe food for consumers. Implementation of food legislation, food quality standard and national food safety programmes such as surveillance, consumers education and food handlers training are critical intervention in the prevention of salmonellosis.

CONCLUSIONS

The presence of *Salmonella* in various food, both animal origin and non-animal origin, indicated that it is a never ending threat to human life. It is also a challenge for food industries and governments to detect it and to improve food safety continously. Implementation of food safety programmes in food industries and intervention by governments through legislation and education are requirement to fight the *Salmonella* and to improve the food safety continously.

ACKNOWLEDGEMENT

The authors thank Widya Mandala Surabaya Catholic University for financial and resources supporting for this research.

REFERENCES

[1] Brands, D. (2006) *Salmonella*: Deadly Diseases and Epidemics. Chelsea House Publishers, Philadelphia. 102p

[2] Forsythe, S.J. and P.R. Hayes (1998) Food Hygiene, Microbiology and HACCP. 3rd edition. Aspen Publishers, Inc., Gaithersburg, Maryland. 449 p

[3] World Health Organization (2009) Food safety and foodborne illness. Available at www.who.int/mediacentre/factsheets/fs237/en/index.html

[4] Mweu, E. and M. English (2008) Thypoid fever in children in Africa. Trop Med Int Health, 13(4):532-540

[5] Nygård, K., B. De Jong, P. J. Guerin, Y. Andersson, A. Olsson and J. Giesecke (2004) Emergence of new *Salmonella enteritidis* phage type in Europe surveillance of infection in returning travellers. BMC Medicine, 2:32

[6] Goh, Y.L., S.D. Puthucheary, R. Chaudhry, Z.A. Bhutta, M. Lesmana, B.A. Oyofo, N.H. Punjabi, A. Ahmed and K.L. Thong (2002) Genetic diversity of *Salmonella enterica* serovar paratyphi A from different geographical regions in Asia. Journal of Applied Microbiology, 92:1167-1171

[7] Berkelman, R.L., M.L. Cohen, J. Yashuk, T. Barrett, J.G. Wells and P.A. Blake (1983) Traveler's diarrhea at sea: two multi-pathogen outbreaks caused by food eaten on shore visits. American Journal of Public Health, 73: 770-773

[8] Harris, J. R., D. Bergmire-Sweat, J.H. Schlegel, K.A. Winpisinger, R.F. Klos, C. Perry, R.V. Tauxe and M.J. Sotir (2009) Multistate outbreak of *Salmonella* infections associated with small turtle exposure, 2007-2008. Pediatrics, 124(5):1388-1394

[9] Hedican, E., C. Hooker, T. Jenkins, C. Medus, S. Jawahi, F. Leano and K. Smith (2009) Restaurant *Salmonella* enteritidis *outbreak* associated with an asymptomatic infected food worker. Journal of Food Protection, 72(11):2332-2336

[10] Sotir, M.J., G. Ewald, A.C. Kimura, J.I. Higa, A. Sheth, S. Troppy, S. Meyer, R.M. Hoekstra, J. Austin, J. Archer, M. Spayne, E.R. Daly, P.M. Griffin (2009) Outbreak of *Salmonella wandsworth* and *typhimurium* infections in infants and toddlers traced to a commercial vegetable-coated snack food. Pediatr Infect Dis J, 28(12):1041-1046

[11] Willis, C., C.L. Little, S. Sagoo, E. de Pinna and J. Threfall (2009) Assessment of the microbiological safety of edible dried seeds from retail premises in the United Kingdom with a focus on *Salmonella* spp. Food Microbiology, 26(8): 847-852

[12] Van Duynhoven, Y.T., L.D. Isken, K. Borgen, M. Besselse, K. Soethoudt, O. Haitsma, B. Mulder, D.W. Notermans, R. De Jonge, P. Kock, W. van Pelt, O. Stenvers and J. van Steenbergen (2009) A prolonged outbreak of *Salmonella typhimurium* infection related to an uncommon vehicle: hard cheese made from raw milk. Epidemiol Infect, 137 (11):1548-57

[13] Irvine, W.N., I.A. Gillespie, F.B. Smyth, P.J. Rooney, A. McClenaghan, M.J. Devine and V.K. Tohani (2009) Investigation of an outbreak of *Salmonella enterica* serovar Newport infection. Epidemiol Infect, 137 (10): 1449-1456

[14] van Cauteren, D., N. J. da Silva, F.X. Weill, L. King, A. Brisabois, G. Delmas, V. Vaillant and H. de Valk (2009) Outbreak of *Salmonella enterica* serotype Muenster infections associated with goat's cheese, France, March 2008. Euro Surveill, 14: 31

[15] Wibowo, D and Ristanto (1988) Petunjuk Khusus Deteksi Mikroba Pangan. Center for Interuniversity, Gadjah Mada University, Yogyakarta, Indonesia. 253 p

[16] Jalal, K.C.A., M. Najiah, M. Fathiyah, Y. Kamaruzzaman, M.N. Omar, S.M. Amir and I. Jaswir [2009] Bacterial pollution in molluscs arch clam, *Orbicularia orbiculata* and blood cockle, *Anadara granosa* of Pahang Estuary, Malaysia. Malaysia J. Biol.Sciences, 9(8): 841-850.

[17] Hatha, M. A. A., T.K. Maqbool and S.S. Kumar (2003) Microbial quality of shrimp products of export trade produced from aquacultured shrimp. Int J Food Microbiol, 82(3):213-221.

[18] Katsuno, S., H. Ando, T. Seo., T. Shinohara, K. Ochiai and M. Ohta (2003) A case of retroperitoneal abcess caused by *Salmonella oranienburg*. Journal of Pediatric Surgery, 38(11):1693-1695.

[19] Wilson, I.G. and J.E. Moore (1996) Presence of *salmonella* spp and *campylobacter* spp in shellfish. Epidemiol Infect, 116(2):147-153.

[20] Martinez-Urtaza, J., M. Saco, J. de Novoa, P. Perez-Pineiro, J. Peiteado, A. Lozano-Leon and O. Garcia-Martin (2004) Influence of environmental factors and human activity on the presence of *salmonella* serovars in a marine environment. Appl Environ Microbiol, 70(4):2089-2097.

[21] Martinez-Urtaza, J., M. Saco, G. Hernandez-Cordova, A. Lozano O. Garcia-Martin and J. Espinosa (2003) Identification of *salmonella* serovar isolated from live molluscan shellfish and their significance in the marine environment. Journal of Food Protection, 70(4):2089-2097.

[22] Monfort, P., D. le Gal, J.C. le Saux, G. Piclet, P. Raquenes, S. Boulben and A. Plusquellec (1994) Improved rapid method for isolation and enumeration of *salmonella* from bivalves using rambach agar. Journal of Microbiological Methods, 19(1):67-79

[23] Correa, A.A., J. Toso, J.D. Albarnaz, C.M.O. Simoes and C.R.M. Barardi (2006) Detection of *Salmonella typhimurium* in oysters by PCR and molecular hybridization. Journal of Food Quality, 29:458-469

[24] Kumar, R., P.K. Surendran and N. Thampuran (2008) Evaluation of culture, ELISA and PCR assays for the detection of *Salmonella* in seafood. Letters in Applied Microbiology, 46:221-226

[25] Purchon, R.D. (1977) The biology of the mollusca. 2nd edition. Pergamon press, Oxford. 560 p

[26] Trihendrokesowo (1987) Penyakit Infeksi Akibat Pangan. Gadjah Mada University Press, Yogyakarta, Indonesia. 211 p

[27] Centers for Disease Control and Prevention (2009) Surveillance for foodborne disease oubreaks-United Sates,2006. Morbidity and Mortality Weekly Report 58:22

[28] Badrinath, P., T. Sundkvist, H. Mahgoub and R. Kent (2004) An outbreak of *Salmonella enteridis* phage type 34a infection associated with a Chinese restaurant in Suffolk, United Kingdom. BMC Public Health, 4:40

[29] Saggers, E.J., C.R. Waspe, M.L. Parker, K.W. Waldron and T.F. Brocklehurst (2008) *Salmonella* must be viable in order to attach to the surface of prepared vegetable tissues. Journal of Applied Microbiology, 105:1239-1245

[30] Long, G.K., G.K. Adak, S.J. O'Brien and I.A. Gillespie (2002) General outbreaks of infectious intestinal disease linked with salad vegetables and fruit, England and Wales, 1992-2000. Commun Dis Public Health, 5:101-105

[31] Naravaneni, R. and K. Jamil (2005) Rapid detection of food-borne pathogens by using molecular techniques. Journal of Medical Microbiology, 54:51-54

[32] Anonim (2009) *Salmonella* detection and identification methods. Available at: http://www.rapidmicrobiology.com/test-methods/Salmonella.php?j=y

[33] Josefsen, M.H., M. Krause, F. Hansen and J. Hoorfar (2007) Optimization of a 12-hour TagMan PCR-based method for detection of *Salmonella* bacteria in meat. Appl Environ Microbiol, 73(9): 3040-3048

[34] Kardos, G., T. Farkas, M. Antal, N. Nogrady and I. Kiss (2007) Letters in Applied Microbiology, 45:421-425

[35] Schneider, A., C. Gronewald, M. Fandke, B. Kutrh, S. Barkowski and K. Berghof-Jager (2002) Real-time detection of the genus Salmonella with the lightcycler system. Biochemica, 4:19-21

[36] Lee, H.A., Wyatt, G.M., S. Bramham and M.R. Morgan (1990) Enzyme-linked immunosorbent assay for *Salmonella typhimurium* in food feasibility of 1-day *Salmonella* detection. Appl Environ Microbiol 56(6)1541-1546

[37] Taban, B.M., U. Ben and S.A. Aytac (2009) Rapid detection of *Salmonella* in milk by combined immunomagnetic separation-polymerase chain reaction assay. J. Dairy Sci, 92:2382-2388